CXXXI. THE ACTION OF CYSTINE AND METHIONINE ON LIVER FAT DEPOSITION

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BEESTON & CHANNON [1936] reported early studies on the question as to whether the preventive action of proteins, such as caseinogen, on fat deposition in the liver was to be attributed to one or more of the constituent amino-acids. Investigation of the action of cystine showed that, so far from preventing fat deposition, this particular amino-acid accelerated it in a remarkable manner. Thus the mean percentage of fat in the livers of 45 control animals receiving a diet containing 40 % fat and 5 % caseinogen was increased from 21.5 to 31.78 by cystine, while the actual weight of fat in the liver of the 100 g. rat rose from 1.08 to 2.02 g., very marked effects being obtained with an intake of 5–7 mg. cystine per rat per day. Further, some extremely high values were encountered in individual animals receiving cystine, the highest being one in which the liver constituted 11.88 % of the body weight and contained 48.96 % of its fresh weight as fat (5.82 g. of fat in the liver of a 100 g. rat).

These results with cystine led naturally to a similar study of methionine which is reported in the present paper. While this work was in progress Tucker & Eckstein [1937] recorded results of studies of both cystine and methionine. Their results with cystine fully confirm those of Beeston & Channon [1936] referred to above. They further observed that methionine in contrast with cystine inhibited liver fat deposition and in consequence suggested that the lipotropic action of any protein depends on the balance between its contents of cystine and methionine. This suggestion is discussed later in this paper.

EXPERIMENTAL

Exp. 1 was carried out in order to compare the actions of cystine and methionine on the "cholesterol" fatty liver. The control group of 9 male rats received the following diet: caseinogen (alcohol-extracted) 5, beef dripping 20, glucose 62, marmite 5, cod liver oil 1, salts 5, cholesterol 2. The two other groups of 7 and 9 animals received the same diet with the addition of 0.2 % cystine and 0.25 % methionine respectively, these two percentages being approximately equimolecular. After 21 days' feeding the animals were guillotined, and the total lipoids extracted from the pooled livers of each group by the alcohol-ether method. Free and total cholesterol, ether-soluble phosphorus, fatty acids and unsaponifiable matter were then determined on the total ethereal extract, and the composition of the liver lipoids calculated as previously described. The results are presented in Table I, from which the lecithin figures have been omitted, since neither in this nor the later experiments did they show changes of interest.

The striking effect of the administration of cystine is shown in Table I where the glyceride has increased from the control figure of 13.24 to 32.65 % of the fresh liver weight. These figures correspond to the presence in the liver of 0.569 g.

Table I. *Exp. 1. The effects of cystine and methionine in "cholesterol" fatty liver production*

Group	Final body wt. g.	Wt. change ± % initial body wt.	Food intake g./rat/day	Liver % final body wt.
Control	195	- 5.5	8.5	4.3
Control + methionine	180	- 11.9	7.7	4.0
Control + cystine	216	0	9.0	6.3

Group	Liver lipoids					
	% fresh liver wt.			g. in liver of 100 g. rat		
	Chole- sterol	Chole- steryl oleate	Glyceride	Chole- sterol	Chole- steryl oleate	Glyceride
Control	0.291	3.197	13.24	0.012	0.137	0.569
Control + methionine	0.289	2.212	10.12	0.012	0.088	0.441
Control + cystine	0.257	3.352	32.65	0.016	0.211	2.057

of glyceride in the control animals, and 2.057 g. in the livers of those receiving cystine. Thus the daily ingestion of only 18.2 mg. cystine per rat has increased the percentage of glyceride to two and a half times, and the absolute weight to nearly four times that of the control animals which is already 10 to 15 times greater than the normal amount. This experiment thus extends the previous finding of the accelerating effect of cystine on the deposition of glyceride in the "fat" fatty liver to that of the "cholesterol" fatty liver.

In clear contrast, methionine has had no effect in increasing the glyceride deposition. In the methionine group the glyceride percentage, 10.12, is actually 3.12 units lower than the control figure of 13.24 and the weight of glyceride in the liver 0.441 is also somewhat less than the 0.569 g. of the control group. While the results suggest that methionine might actually have had a slight effect in decreasing the liver fat, our previous experience of the variations which are to be met with even in groups of 10 animals are such as to make this deduction unwarranted. From this experiment we concluded, therefore, that a methionine intake of 19.25 mg. per rat per day had no effect, whereas a cystine intake of 18.2 mg. strikingly increased the amount of glyceride in the livers [Beeston *et al.* 1937].

The next experiment was carried out on animals receiving a diet calculated to produce "fat" fatty livers. Four groups of ten animals were used. The two control groups received the following diet: caseinogen (alcohol-extracted) 5, beef dripping 40, cod liver oil 1, glucose 49, inorganic salts 5, aneurin 10 μ g. per rat per day. The two other groups received the same diets supplemented with 0.25 and 0.5 % of methionine respectively dissolved in the water used in the preparation of the diets. The animals received this diet for 16 days, and the unsaponifiable matter and fatty acids ("fat" of Table II) were extracted from each liver after saponification, firstly by aqueous, followed by alcoholic NaOH. The results are recorded in Table II as mean values, the extreme values being quoted in brackets.

Table II shows that the percentages of fat in the livers of the two control groups are 16.4 and 13.21, corresponding to 0.677 and 0.509 g. of fat respectively in the liver of the 100 g. rat. As previously pointed out, variations of this magnitude are to be expected in experiments of this type. Both the percentages of fat in the liver, 15.16 and 12.53, and the weight of fat in the liver of the 100 g. rat of the two groups of animals receiving methionine, 0.535 and 0.503 g., are in our

Table II. *Exp. 2. The effect of methionine in "fat" fatty liver production*

Group	No. of animals	Initial body wt. g.	Wt. loss % initial body wt.	Liver % body wt.	Fat % liver	Fat in liver of 100 g. rat g.
Control 1 Extremes	10	167	- 11.0	3.91 (5.64-3.28)	16.40 (33.40-6.70)	0.677 (1.8860-0.2198)
Control 2 Extremes	9	168	- 9.4	3.86 (4.39-3.34)	13.21 (17.71-9.23)	0.509 (0.7101-0.3493)
Methionine 0.25 % Extremes	10	167	- 6.2	4.06 (4.89-3.45)	15.16 (27.63-12.66)	0.535 (1.4340-0.4220)
Methionine 0.5 % Extremes	10	166	- 5.8	3.96 (5.28-3.15)	12.53 (22.62-6.33)	0.503 (1.0201-0.2597)

experience not significantly different from those in the controls. The food intake in this experiment was not measured but in experiments on this diet by animals of this weight it is usually about 8 g. per rat per day. This would correspond to the intake of 20 and 40 mg. of methionine per rat per day in the two groups. The results of this experiment, therefore, are similar to those of Exp. 1 in showing that, in contrast to cystine, methionine at a considerably greater daily intake has had no effect on liver fat deposition. At this point the paper by Tucker & Eckstein [1937] appeared, in which, in contrast to the three results of Exps. 1 and 2, they recorded results showing that methionine had a marked action in decreasing fat deposition. Inspection of their data showed that the amount of "fat" in the livers of their control groups was 19-20% as compared with 13-16% of the three groups of Exps. 1 and 2 of the present work. This suggested an explanation of these divergent findings, for it is our general experience that the ease of prevention of fat deposition in the liver by a given substance depends on the amount which would have been present in the absence of that substance from the diet. The next two experiments were therefore carried out under conditions calculated to produce much more fatty livers, and for this purpose, acting on previous experience, albumin was substituted for caseinogen in order to increase the glyceride fraction. In Exp. 3, designed to produce the "fat" fatty liver, the control group of animals received a diet consisting of egg albumin 5, beef dripping 40, cod liver oil 1, glucose 49, salt mixture 5 parts. In Exp. 4 the control group received egg albumin 5, beef dripping 30, cod liver oil 1, cholesterol 2, glucose 57 and salt mixture 5 parts. The methionine groups in each experiment received the same diets with the addition of 0.5% methionine. Further, all the animals received 10 μ g. aneurin per day. In Exp. 3, the individual livers were worked up as described for Exp. 2, and in Exp. 4 the pooled livers were analysed as described for Exp. 1. The results are recorded in Tables III and IV.

Table III shows that the "fat" percentage of the livers of the methionine group, 14.06, is 9.55 units less than that of the control group, 23.61, with a corresponding decrease in the weight of fat from 1.460 to 0.6262 g. The glyceride fraction in the "cholesterol" fatty livers of Exp. 4 shows a considerable decrease

Table III. *Exp. 3. The effect of methionine in "fat" fatty liver prevention*

Group	No. of animals	Initial body wt. g.	Wt. loss %	Liver % final body wt.	"Fat" % fresh liver	Fat in liver of 100 g. rat g.
Control Extremes	9	169	5.4	5.54 (3.81-8.27)	23.61 (10.68-40.05)	1.460 (0.4067-3.3120)
Control + methionine Extremes	9	169	9.2	4.29 (3.57-5.03)	14.06 (7.03-18.9)	0.6262 (0.2519-0.9500)

Table IV. *Exp. 4. The effect of methionine in the prevention of glyceride deposition in "cholesterol" fatty livers*

Group	No. of animals	Average initial body wt. g.	Wt. change $\pm$ % initial body wt.	Liver % final body wt.
Control	10	172	- 8.9	6.89
Control + methionine	10	171	- 8.8	5.09

Liver lipoids						
Group	% fresh liver wt.			g. in liver of 100 g. rat		
	Cholesterol	Cholesteryl oleate	Glyceride	Cholesterol	Cholesteryl oleate	Glyceride
Control	0.262	1.673	33.78	0.018	0.194	2.327
Control + methionine	0.244	1.278	19.64	0.012	0.109	1.000

also, from 33.78 to 19.64 % and from 2.327 to 1.000 g. Both Exps. 3 and 4 therefore confirm the findings of Tucker & Eckstein that methionine has a preventive action on glyceride deposition in the liver when fed in the concentration used by them, 0.5 %, and when the glyceride percentage in the liver is sufficiently high. Evidence concerning the relative magnitudes of this action and that of choline was then sought in Exp. 5 on the "cholesterol" fatty liver.

In this experiment two groups of animals received the same diets as were used in Exp. 4, i.e. the "cholesterol" fatty liver-producing diet with 8 % albumin as the protein with and without 0.5 % methionine. A third group received the control diet with the addition of 0.04 % choline chloride. Two other groups were also used. The fourth group received the control diet with the substitution of 8 % caseinogen for the 8 % albumin, while the fifth received this caseinogen diet with the addition of 0.5 % methionine. A group receiving both caseinogen and choline chloride was not included, since we have already sufficient data concerning the action of choline on the "cholesterol" fatty liver when caseinogen is the protein of the basal diet. All the groups contained 10 animals

Table V. *Exp. 5. Preventive effect of methionine on the "cholesterol" fatty liver*

Group	Average initial body wt. g.	Wt. change ± % initial body wt.	Liver % final body wt.
8% albumin	169	-3	7.80
8% albumin + 0.04% choline chloride	167	+1	5.86
8% albumin + 0.5% methionine	164	+3	5.09
8% caseinogen	164	-7	6.63
8% caseinogen + 0.5% methionine	164	0	4.53

Group	Liver lipoids					
	% fresh liver wt.			g. in liver of 100 g. rat		
	Chole- sterol	Chole- steryl oleate	Glyceride	Chole- sterol	Chole- steryl oleate	Glyceride
8% albumin	0.31	4.55	36.20	0.024	0.355	2.82
8% albumin + 0.04% choline chloride	0.32	5.47	27.14	0.019	0.321	1.59
8% albumin + 0.5% methionine	0.29	3.97	21.30	0.015	0.200	1.08
8% caseinogen	0.34	4.98	32.19	0.023	0.330	2.13
8% caseinogen + 0.5% methionine	0.30	3.44	17.79	0.014	0.156	0.81

which were fed for 21 days, when the pooled livers were completely analysed as in Exp. 1. The data are presented in Table V.

Confining attention for the moment to the glyceride fraction, it is seen that in both the control albumin and caseinogen groups very high figures resulted, 36.20 and 32.19 % and 2.82 and 2.13 g. respectively. The inclusion of 0.5 % methionine has reduced each of these control figures by some 15 units %, the decreases in weight being 1.8 and 1.3 g. respectively. These results show that methionine has had a greater effect on the livers of the albumin group than on those of the caseinogen group. This may be connected with the greater fat content of the livers of the former, but the nature of the protein may also be involved. The livers of the animals receiving 0.04 % choline chloride with 8 % albumin contain 27.14 % and 1.59 g. per 100 g. rat as compared with 36.20 and 2.82 g. of the albumin group. These reductions are about 60 % of that caused by 0.5 % methionine.

DISCUSSION

The results of Exps. 3-5, which are in effect four separate observations, show clearly that the inclusion of 0.5 % methionine in a diet will prevent glyceride deposition in the livers of rats receiving diets high in fat with or without added cholesterol. The results of these experiments in which the control diet has caused the appearance of more than 20 % glyceride in the liver, confirm those of Tucker & Eckstein [1937]. Those of Tables I and II, consisting of three experiments, show equally clearly the lack of effect of 0.25 % methionine in two experiments, and 0.5 % in a third, when the control glyceride percentage is below 16 %. From the present results and from much previous work on the action of choline, it is clear that the action of methionine is far less intense than is that of choline itself, and it is of value to attempt to deduce something of the magnitude of its action. In all the experiments in which an action has been observed the diet has contained 0.5 % methionine, corresponding to an intake of about 40 mg. per rat per day. It is this figure, therefore, which must be equated with choline. In the albumin groups of Exp. 5 a daily intake of about 3 mg. choline has had about 60 % of the action of this amount of methionine, but there still remains in the liver a very high percentage of glyceride, 21.3 %. It seems reasonable in the light of previous experience to believe that 4-6 mg. choline would have had an effect equal to that of 40 mg. methionine in this experiment. Comparison may also be made with the action of choline in experiments carried out for other purposes. In any such indirect comparison, it is necessary that the level of glyceride be approximately the same as that which occurred in the control groups of the methionine experiments, and in the present state of knowledge it appears desirable that only experiments in which the same protein was used be compared. There are accordingly recorded in Table VI unpublished data for comparison with those of Exps. 3 and 4 and the second part of Exp. 5.

The percentage and weight of fat in Exp. (a) of Table VI are very similar to those of Exp. 3, the 2.2 mg. choline having had a rather greater effect than the 40 mg. methionine in that experiment. Accepting the actions as identical, the effect of methionine is $\frac{1}{18}$ that of choline. In Exp. (b), Table VI, the figures are also very similar to those of Exp. 4, indicating methionine to have $\frac{1}{20}$ of the activity of choline. In Exp. (c), Table VI, 4.1 mg. choline have had an action similar to the 40 mg. methionine of the second part of Exp. 5. This experiment also shows the effect of increasing the choline intake to 9.2 mg., for the percentage and weight of glyceride fall well below that caused by methionine in Exp. 5. The results of the first part of Exp. 5 and of the three comparisons just recorded indicate the activity of methionine to lie between $\frac{1}{8}$ and $\frac{1}{20}$ that of

Table VI

	Fat in liver %	Fat in liver of 100 g. rat g.	
	"Fat" fatty liver		
(a) 8% albumin	25.95	1.467	cf. Exp. 3, Table III
8% albumin + 2.2 mg. choline/rat/day	13.30	0.532	
	Glyceride in liver %	Glyceride in liver of 100 g. rat g.	
	"Cholesterol" fatty liver		
(b) 8% albumin	36.58	3.106	cf. Exp. 4, Table IV
8% albumin + 2 mg. choline/rat/day	25.66	1.506	
(c) 5% caseinogen	31.30	1.960	cf. Exp. 5, part 2, Table V
5% caseinogen + 4.1 mg. choline/rat/day	13.73	0.630	
5% caseinogen + 9.2 mg. choline/rat/day	7.44	0.294	

choline. This low activity of methionine explains our failure to observe any effect of this amino-acid in Exps. 1 and 2.

The results of this work and of that of Beeston & Channon [1936] thus provide an interesting illustration of the opposing action of these two amino-acids, cystine in enormously accelerating fat deposition in the liver and methionine in exerting a preventive action of less striking character. These facts are of particular significance in view of the growing body of evidence indicating that cystine and methionine do not follow the common metabolic path assigned to them by Jackson & Block [1932] [cf. Brand *et al.* 1935; Lewis *et al.* 1936; Rose, 1937; Rose *et al.* 1937; White & Beach, 1937]. In this connexion the actions of S-methylcysteine and homocystine on liver fat are being investigated in this laboratory.

Since the original observations of Beeston & Channon [1936] on the accelerating action of cystine in fat deposition in the liver, it has been obvious that the action of proteins in preventing fat deposition—the so-called lipotropic action—must be the result of a balance between factors accelerating and preventing fat deposition, provided that amino-acids liberated from proteins during digestion behave in the same way as amino-acids added to a diet. The observations on methionine recorded here and those of Tucker & Eckstein [1937] provide the first example of an amino-acid decreasing liver fat. The latter authors have in fact made the suggestion that the lipotropic action of any protein depends on its relative cystine and methionine contents. In this connexion it is to be noted that the effect of the daily intake by the rat of 0.5 g. caseinogen can be readily seen in the decrease in the amount of fat in its liver. This amount of caseinogen would contain about 17 mg. methionine, which on the evidence presented in this paper would be equivalent in its action to, say 1.5 mg. choline. While amounts of choline of this order may have a marked action depending on the percentage of fat which would occur in the liver in their absence from the diet and possibly on the nature of the dietary protein, it seems to us desirable to await considerable further evidence before a judgment can be made as to whether the methionine and cystine contents of proteins account entirely for their lipotropic action. The completion of our studies on the effect of the different amino-acids, and work which is in progress in which pure proteins of different cystine and methionine contents are being supplemented with these amino-acids in order to compare them from this point of view, will provide such evidence. Further, it has yet to be established that the lipotropic action of

proteins is due to one or more constituent parts of the molecule, and not to contaminants.

Attention may be drawn to the fact that the administration of methionine has decreased the amount of cholesteryl ester present in the liver of the 100 g. rat in all the experiments in which cholesterol was employed, i.e. Exps. 1 and 4, and both parts of Exp. 5, the decreases in Exps. 4 and 5 being of the order of 50 % of the control value. The decrease in Exp. 1, in which the amount of cholesteryl oleate, 0.088 g., is little more than 60 % of the control value, 0.137 g., is particularly interesting since methionine caused no decrease in the glyceride fraction in this experiment. In all these experiments, save Exp. 1, there has also been a decrease in the amount of free cholesterol in the livers. These decreases in cholesterol and cholesteryl esters also occur when choline is administered, although we have not observed in a large range of experiments an effect on the cholesterol fractions without an effect on the glyceride fractions.

SUMMARY

1. A study has been made of the effect of the administration of methionine to rats receiving low protein diets of high fat content with and without added cholesterol, on lipid deposition in the liver.

2. In two experiments on the "fat" fatty liver, an intake of 20 and 40 mg. methionine per rat per day had no preventive effect when the "fat" in the livers of the control animals was 16.4 and 13.2 %. In a third experiment 40 mg. had a marked preventive action when the fat in the liver was 23.6 %.

3. Similar results were obtained in experiments on the "cholesterol" fatty liver. In one experiment 20 mg. methionine had no effect when the glyceride percentage in the control groups was 13.2 %. In three further experiments, in which the control glyceride percentage was greater than 20, 40 mg. methionine had a considerable preventive action.

4. Evidence is adduced that the intensity of action of methionine in preventing glyceride deposition in the liver is about $\frac{1}{12}$ that of choline.

5. The striking effect of the administration of small amounts of cystine in increasing the deposition of glyceride in "cholesterol" fatty livers is demonstrated.

6. The administration of methionine with cholesterol-containing diets markedly decreases the deposition of cholesterol, both free and as ester.

7. The bearing of these findings of the antagonistic actions of methionine and cystine on the problem of metabolism of these amino-acids and on the possibility that the lipotropic action of proteins may be accounted for by this action is discussed.

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